Dept. Genetics,
University of Wisconsin.
October 6, 1948.

Dear Jacques,

Thank you for your reprint on adaptation. I hope that the cultures I sent you will have reached you, and perhaps been of some use.

Using o-nitrophenyl b-galactoside as a chromogenic substrate, I have been continuing my work on coli Lactase. The substrate has proven to be a very useful tool. Cell free extracts of very high potency have been readily obtained from K-12 with the Booth-Green Mill (thanks to Dr. Green, who is on our campus now), but nearly comparable activity can be gotten out by autolysis overnight under toluene. Prolonged autolysis leads to diminution of recovery.

Two interesting points have come up in relation to the "isolated" enzyme. Firstly, is a requirement for, or rather a strong stimulation by Na ion. Preparations made up in phosphate or glucerophosphate buffer are stimulated up to 100% by M/50 NaCl or Na_2SO_4. The other alkali metals and NH_4 are either inert or inhibitory (competitive?). A requirement for phosphate has been rigorously excluded (unless $< 10^{-5}$ or less is already optimal) by tests in barbital and in glycerophosphate buffer, and analyses for inorganic phosphate. Secondly is the inhibition not only by lactose, but by every reducing sugar I have tried, of the enzyme.(with respect to its activity on the galactoside). Sugars not utilized by the organism are equally inhibitory (e.g. cellobiose) and it makes no difference whether the cells are Mal- or Mal+ for inhibition my maltose. Sucrose and trehalose are inert (the first utilizable; the second not). This would seem to suggest that the capacity to bind with the enzyme and block its function is relatively non-specific, while the specificty is required to split the substrate. The preparations show good linear responses, extrapplating to the origin both for time vs. product formed, and for product formed vs. enzyme conc., strongly supporting the unitary character of the enzyme. The enzyme is formed only in lactose (or other galactoside) adapted cells of wild type, and none of the several genetic types of Lac- so far tested shows any signs of lactase production. All of which further tends to support the notion of a complex kind of gene enzyme relationship.

My work on this material has been somewhat diverted by the discovery of heterozygosis in prototrophs from crosses of certain K-12 stocks. As you know, the prototrophs are typically pureline and are assumed to have segregated already from the previous zygote. A stock has been found (spontaneous mutant,in 53-161 ?) which in crosses with standard stocks gives Lac+ prototrophs which are not stable, but when put on complete, EMB Lac medium, segregate out into Lac- and Lac+ types, which are of all possible recombination classes, including the hitherto elusive multiple mutant class, (i.e. B- L-T-Lac- etc...) This interesting phenomenon seems to be associated with an aberration in the region of the Mal$_1$ locus, but hasn't been thoroughly worked out.

Another item that may be of interest to you is a new method for isolating biochemic mutants which has worked very successfully so far, primarily with Salmonella. It depends on the fact that penicillin (300 OU/ml for coli) is bactericidal only for growing cells. If an irradiated suspension is washed and inoculated into a synthetic medium with penicillin and incubated 4-6 hours, the growing, non-mutant cells are killed preferentially, amplifying the proportion of mutants among the survivors. Amplification of 10^3 and higher have been found in reconstruction experiments with coli, and with Salmonella, runs in which ½ the colonies fail to grow on minimal on first test are typical. So far, no evidence of recombination in Salmonella.

With best regards

Yours sincerely,